Analysis of Ethylene Glycol from Blood

1 Introduction

Ethylene glycol (EG) is a toxic glycol used in coolants and antifreezes. It causes central nervous system depression similar to that of ethanol. It is metabolized in the body to oxalic acid, which is damaging to the kidney.

2 Scope

This procedure allows for the qualitative and quantitative analysis of blood samples for EG. This procedure applies to Chemistry Unit case working personnel who perform toxicology analyses.

3 Principle

After addition of an internal standard, blood specimens are mixed with phenylboronic acid in acetone. A small amount of the acetone layer is added to a headspace vial which is crimped. The vial is thermostatted to cause total volatilization of the acetone and ethylene glycol derivative. The headspace is analyzed by HS-GC/MS(EI) (headspace gas chromatography mass spectrometry in electron impact mode).

4 Specimens

0.1 mL of blood is required for each replicate; quantitative analyses are performed in duplicate.

5 Equipment/Materials/Reagents

a. Gas Chromatograph / Mass Spectrometer (GC/MS) with a headspace autosampler capable of EI ionization and equipped with a 30 m x 0.25 mm x 0.25 µm film thickness DB-5 (or equivalent) column

b. Vortex mixer

c. Centrifuge

d. Volumetric pipets with appropriate tips

e. Routine laboratory supplies, including centrifuge tubes with caps, 10 cc headspace vials with caps, etc.

f. Phenylboronic acid (HPLC grade)

g. Acetone (HPLC grade)

h. Methanol (HPLC grade)

i. Phenylboronic Acid Derivatizing Reagent (5 mg/mL):
Add 125 mg of phenylboronic acid to a 25-mL volumetric flask. Dilute to the mark with acetone and mix well. Store at room temperature in glass. Stable for at least one month.

6 Standards and Controls

a. Ethylene glycol (EG) Stock Standard (10 mg/mL):
For quantitation, two sources of ethylene glycol should be obtained. Ethylene glycol traceable to United States Pharmacopoeia (USP) can be purchased from USP or another approved vendor. Storage and stability are determined by the manufacturer. Add 100 mg of EG to a 10-mL volumetric flask. Bring to the mark with methanol and mix well to dissolve. Store refrigerated in glass; stable for at least 1 year.

b. EG Calibrator Working Stock - High (2000 µg/mL):
Add 1.0 mL of the EG Stock Standard (10 mg/mL) to a 5-mL volumetric flask. Bring to the mark with methanol and mix well to dissolve. Store refrigerated in glass; stable for at least 1 year.

c. EG Calibrator Working Stock - Low (200 µg/mL):
Add 0.2 mL of the EG Stock Standard (10 mg/mL) to a 10-mL volumetric flask. Bring to the mark with methanol and mix well to dissolve. Store refrigerated in glass; stable for at least 1 year.

d. EG Control Working Stock - High (2000 µg/mL):
Add 1.0 mL of the EG Stock Standard (10 mg/mL; different source than that used to make the Calibrator Working Stock) to a 5-mL volumetric flask. Bring to the mark with methanol and mix well to dissolve. Store refrigerated in glass; stable for at least 1 year.

e. EG Control Working Stock - Low (200 µg/mL):
Add 0.2 mL of the EG Calibrator Stock Standard (10 mg/mL; different source than that used to make the Calibrator Working Stock) to 10-mL volumetric flask. Bring to the mark with methanol and mix well to dissolve. Store refrigerated in glass; stable for at least 1 year.

f. Negative Control Blood:
Purchased from Cliniqa or another approved vendor. Storage and stability are determined by the manufacturer.

A Negative Control Blood sample is analyzed with every blood assay.

g. d_4-Ethylene glycol (d_4-EG) Internal Standard Working Standard (400 µg/mL):
d_4-Ethylene glycol (98% or better) can be purchased from Isotech or another approved vendor.

Storage and stability are determined by the manufacturer. Add 9 µL of d_4-EG to a 25-mL volumetric flask. Bring to the mark with methanol and mix well to dissolve. Store refrigerated in glass; stable for at least 1 year.

h. Calibrator Sample Preparation:
On the day of analysis, add EG and makeup methanol to 0.1 mL blood as described in the table below in order to generate a 6 point calibration curve:

Calibrator Level	EG Conc (µg/mL)	EG Calibrator Working Stock Conc (µg/mL)	Volume EG Calibrator Working Stock (µL)	Volume Makeup Methanol (µL)
1	40	200 (low)	20	70
2	300	2000 (high)	15	75
3	600		30	60
4	1000		50	40
5	1400		70	20
6	1800		90	0

i. Positive Control Sample Preparation:
On the day of analysis, have a second chemist add EG and makeup methanol to 0.1 mL blood as described in the table below in order to create low and high positive controls:

Control Level	EG Conc (µg/mL)	EG Control Working Stock Conc (µg/mL)	Volume EG Control Working Stock (µL)	Volume Makeup Methanol (µL)
1	120	200 (low)	60	30
2	1500	2000 (high)	75	15

Two levels of Positive Control Blood are analyzed in duplicate with each quantitative assay.

j. Qualitative Analysis:
For qualitative analysis, a negative control (f), and one Level 1 and one Level 2 Positive Control (i) will be analyzed along with unknown samples.

k. System Suitability Check:
An additional Level 1 control will be analyzed prior to unknown analysis to verify instrument performance.

7 Sampling

Not applicable.

8 Procedure

Appendix 1 contains an abbreviated version of this procedure that may be used at the bench by the chemist performing the procedure.

a. Label tubes for each sample, calibrator and control.

b. Add 0.1 mL of blood to a snap-cap centrifuge tube. Case samples are prepared in duplicate for quantitative analysis. Additionally, prepare a negative control and calibrator samples as described in Section 6. Have a second analyst prepare positive control samples as described in Section 6.

c. Add 90 µL of makeup methanol to all case samples and negative control(s).

d. Add 25 µL of d_4-EG Internal Standard Solution (400 µg/mL) to each case sample, calibrator and control.

e. Add 400 µL of Phenylboronic Acid Derivatizing Reagent.

f. Cap each tube and vortex-mix for 10 seconds.

g. Centrifuge at 10,000 rpm for 3 minutes.

h. Remove 20 µL of the acetone layer to a labeled 10 cc headspace vial and immediately cap.

i. Analyze by HS-GC/MS(EI) after verifying instrument performance

9 Instrumental Conditions

Appendix 2 contains an abbreviated version of the instrumental conditions that may be used at the bench by the chemist performing the procedure.

9.1 Headspace Sampler Parameters

incubation temperature	125°C	syringe temperature	145°C
incubation time	5 min	injection volume	0.1 mL
agitator speed	300 RPM	sample fill rate	0.050 mL/sec
agitation timing	10 sec on 1 sec off	sample fill strokes	3
use syringe dedicated to high temperatures		sample injection speed	0.25 mL/sec
		syringe flush time	300 sec

9.2 GC Parameters

Oven Parameters		Inlet and Carrier Parameters		Column Parameters	
temperature 1	50°C	inlet temperature	250°C	type	DB5MS
hold 1	0 min	injection mode	Split	length	30 m
ramp 1	20°C/min	split	20:1	internal diameter	0.25 mm
temperature 2	120°C	carrier gas	helium	film thickness	0.25 µm
ramp 2	60°C/min	carrier mode	constant flow		
temperature 3	280°C	carrier flow	1.2 mL/min		
hold 2	3.84 min				

9.3 Mass Spectrometer Parameters

ionization mode	electron impact (+)	source temperature	230°C
scan mode	full scan	transfer line temperature	280°C
scan range	35 - 200 m/z	quad temperature	150°C
		solvent delay	4.0 min

10 Decision Criteria

The following criteria are used as guidelines in determining the acceptability of the data produced in this procedure. In general, compound identification will be based on comparison of the chromatography and mass spectrometry for the Calibrator Sample or Positive Control. In most cases, all of the below should be met in order to identify ethylene glycol within a biological specimen.

10.1 Chromatography

The peak of interest should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. In order to be determined acceptable, a chromatographic peak in an unknown sample should compare favorably to a chromatographic peak in a known sample analyzed on the same system in the same analytical mns. Additionally, the following two criteria should be met.

10.1.1 Retention Time

The retention time of the peak should be within ±2% of the retention time (relative or absolute) obtained from injection of an extracted Positive Control.

10.1.2 Signal-to-Noise

To justify the existence of a peak, its baseline signal to peak-to-peak noise ratio should exceed 3. Further, the baseline signal for the peak from the sample of interest should be at least 10-fold greater than that for any observed peak at a similar retention time in a Negative Control or solvent blank injected just prior to that sample.

10.2 Mass Spectrometry

The following ions are characteristic of the phenylboronic acid derivative of ethylene glycol: 148, 118, and 91. The mass spectrum of the derivatized ethylene glycol should match that of an extracted Positive Control or calibrator. See the *Guidelines for Comparison of Mass Spectra* standard operating procedure (Tox 104) for further guidance.

11 Calculations

Ethylene glycol is quantitated by calculating the area of derivatized EG to the area of its internal standard (148:152) and plotting these ratios against concentration. Linear regression is used to find the best fit line through the data using 1/x weighting. For additional guidance in performing quantitations, see the *Guidelines for Toxicological Quantitations* standard operating procedure (Tox 101).

Results in this method are calculated in the units µg/mL. Results can also be reported in mg/dL. In order to convert from µg/mL to mg/dL, the decimal place is moved once to the left. For example, 500 µg/mL = 50.0 mg/dL.

12 Measurement Uncertainty

The critical sources of measurement uncertainty in this procedure include:

- historical random uncertainty of repeated measurements
- accuracy of the pipette used to deliver the sample
- accuracy of the pipette used to deliver the calibrators
- uncertainty in the concentration of the calibration standards
- precision of the delivery of internal standard

The measurement uncertainty will be estimated and reported following the *Chemistry Unit Procedures for Estimating Uncertainty in Reported Quantitative Measurements* standard operating procedure (CUQA 13). Information used to derive uncertainty measurements will be tracked in an electronic database.

13 Limitations

a. Accuracy: Range of -2.73% to +0.50% at three measured concentrations

b. Calibration Range: 40 – 1800 µg/mL

c. Limit of Detection: 40 µg/mL

d. Precision: Range of 8.73% – 11.24% at three measured concentrations

e. Processed Sample Stability: Not thoroughly evaluated; samples should be analyzed on the day of preparation.

f. Interferences: None known. Laboratory experiments have demonstrated that diethylene glycol, triethylene glycol, propylene glycol, glycerol and 1,4-butanediol do not interfere with the method.

14 Safety

Take standard precautions for the handling of chemicals and biological materials. Refer to the *FBI Laboratory Safety Manual* for guidance.

15 References

Guidelines for Comparison of Mass Spectra (Tox 104); FBI Laboratory Chemistry Unit – Toxicology SOP Manual.

Quality Control for Toxicology Examinations (Tox 101); FBI Laboratory Chemistry Unit – Toxicology SOP Manual.

Chemistry Unit Procedures for Estimating Measurement Uncertainty (CUQA 13); FBI Laboratory Chemistry Unit Quality Assurance and Operations Manual.

FBI Laboratory Safety Manual.

A. Ehlers, C. Morris and M.D. Krasowski. “A rapid analysis of plasma/serum ethylene and propylene glycol by headspace gas chromatography”. *SpringerPlus*, 2 (2013) 1-6.

T. Hlozek, M. Bursova and R. Cabala. “Fast determination of ethylene glycol, 1,2-propylene glycol and glycolic acid in blood serum and urine for emergency and clinical toxicology by GC-FID”. *Talanta*, 130 (2014) 470-414.

W.H. Porter and A. Auansakul. “Gas-chromatographic determination of ethylene glycol in serum”. *Clinical Chemistry*, 28 (1982) 75-78.

R.H. Williams, S.M. Shah, J.A. Maggiore and T.B. Erickson. “Simultaneous detection and quantitation of diethylene glycol, ethylene glycol, and the toxic alcohols in serum using capillary column gas chromatography”. *Journal of Analytical Toxicology*, 24 (2000) 621-626.

Rev. #	Issue Date	History
0	06/03/16	New document
1	11/15/16	Updated internal standard preparation in Section 6g. Subsequently updated step 8d and bench sheet. Updated syringe flush time in Table 9.1.
2	01/10/19	To account for qualitative analysis, the Title and Sections 1, 2, and 4 were updated. Updated Section 2 scope statement. Corrected typo at 5i (ug to mg). 6j was added. Added a system suitability check to 6k. Added 8g centrifuge step. Added clarification to 8i. Removed “subunit” from header, signature lines, and references.

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Approval

Chemistry Unit Chief: Date: 01/09/2019

Tox Technical Leader: Date: 01/09/2019

QA Approval

Quality Manager: Date: 01/09/2019

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